Running title: Molecular phylogeny of *Oreochromis* cichlid fishes

Molecular phylogeny of *Oreochromis* (Cichlidae: Oreochromini) reveals mito-nuclear discordance and multiple colonisation of adverse aquatic environments

Antonia G. P. Ford[a,b], Thomas R. Bullen[a], Longson Pang[a], Martin J. Genner[c], Roger Bills[d], Tomáš Flouri[a], Benjamin P. Ngatunga[e], Lukas Rüber[f,g], Ulrich K. Schliewen[h], Ole Seehausen[g,i], Asilatu Shechonge[e,j], Melanie L. J. Stiassny[k], George F. Turner[l], Julia J. Day[a*]

[a] Department of Genetics, Evolution and Environment, University College London, Gower Street, London, WC1E 6BT, U.K.

[b] Current address: Department of Life Sciences, Whitelands College, University of Roehampton, Holybourne Avenue, London, SW15 4JD, U.K.

[c] School of Biological Sciences, University of Bristol, Life Sciences Building, 24 Tyndall Avenue, Bristol. BS8 1TQ, U.K.

[d] South African Institute for Aquatic Biodiversity, Private Bag 1015, 6140 Grahamstown, South Africa

[e] Tanzania Fisheries Research Institute (TAFIRI) PO. Box 9750. Dar es Salaam. Tanzania

[f] Naturhistorisches Museum Bern, Bernastrasse 15, 3005 Bern, Switzerland

[G] Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

[h] Bavarian State Collection of Zoology, Department of Ichthyology, Münchhausenstr. 21, 81247 München, Germany

[i] Department of Fish Ecology and Evolution, Center for Ecology, Evolution and Biogeochemistry, EAWAG Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland

[j] Department of Aquatic Sciences and Fisheries, University of Dar es Salaam, P.O. Box 35064, Dar es Salaam, Tanzania

[k] Department of Ichthyology, American Museum of Natural History, New York, NY, USA

[l] School of Biological Sciences, Bangor University, Bangor, LL57 2UW, UK.

[*] E-mail address: j.day@ucl.ac.uk, antonia.ford@roehampton.ac.uk

Abstract

Although the majority of cichlid diversity occurs in the African Great Lakes, these fish have also diversified across the African continent. Such continental radiations, occurring in both rivers and lakes have received far less attention than lacustrine radiations despite some members, such as the oreochromine cichlids (commonly referred to as 'tilapia'), having significant scientific and socio-economic importance both within and beyond their native range. Unique among cichlids, several species of the genus *Oreochromis* exhibit adaptation to soda conditions (including tolerance of elevated temperatures and salinity), which are of interest from evolutionary biology research and aquaculture perspectives. Questions remain regarding the factors facilitating the diversification of this group, which to date have not been addressed within a phylogenetic framework. Here we present the first comprehensive (32/37 described species) multi-marker molecular phylogeny of *Oreochromis* and closely related *Alcolapia*, based on mitochondrial (1583 bp) and nuclear (3092 bp) sequence data. We show widespread discordance between nuclear DNA and mitochondrial DNA trees. This could be the result of incomplete lineage sorting and/or introgression in mitochondrial loci, although we didn't find a strong signal for the latter. Based on our nuclear phylogeny we demonstrate that adaptation to adverse conditions (elevated salinity, temperature, or alkalinity) has occurred multiple times within *Oreochromis*, but that adaptation to extreme (soda) conditions (high salinity, temperature, and alkalinity) has likely arisen once in the lineage leading to *O. amphimelas* and *Alcolapia*. We also show *Alcolapia* is nested within *Oreochromis*, which is in agreement with previous studies, and here revise the taxonomy to synonymise the genus in *Oreochromis*, retaining the designation as subgenus *Oreochromis* (*Alcolapia*).

Keywords: Tilapia; *Alcolapia*; ancestral state reconstruction; mito-nuclear discordance; introgression; incomplete lineage sorting; taxonomic revision.

Introduction

The propensity for African cichlids to form adaptive radiations within lacustrine environments has received considerable research attention (Turner 2007, Seehausen 2015), but the processes promoting diversification within largely riverine lineages are less well known. One of the most species rich and widely distributed lineages of African cichlids is the oreochromine group. Oreochromine cichlids have significant scientific and socio-economic importance both within and beyond their native range – providing a major food source for fisheries and aquaculture, a biocontrol agent for aquatic plants and, together with other cichlid groups, they are a 'model' system for evolutionary research (Kobayashi et al. 2015; Yue et al. 2016; Brawand et al. 2014). However, the phylogenetic relationships of the species of the most diverse group - *Oreochromis* Günther 1889 - are poorly understood.

As defined by Dunz and Schliewen (2013), the tribe Oreochromini is comprised of the mouthbrooding lineages formerly assigned to the tilapiine tribe: *Alcolapia, Danakilia, Iranocichla, Oreochromis, Sarotherodon, Tristramella,* along with four *Sarotherodon*-derived genera endemic to the Cameroonian crater Lake Barombi Mbo (*Konia, Myaka, Pungu, Stomatepia*). The most diverse genus, *Oreochromis*, is found throughout Sub-Saharan Africa, as well as the Nile basin and Middle East (Trewavas 1983). The Oreochromini largely occur in rivers, floodplains and shallow lakes (75% of species occur in rivers, or rivers and lakes; Trewavas 1983) and while members occur in every larger lake of Africa (and in many small ones), they have only formed low diversity radiations in very few lakes (Lowe-McConnell 1959; Trewavas 1982; Klett & Meyer 2002; Seehausen 2007), and these usually are lakes that lack haplochromine cichlids (Seehausen 2007).

Oreochromine cichlids are attractive targets for aquaculture, with at least eight species actively farmed globally. The Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed aquaculture species globally, but other species of *Oreochromis* are farmed in many local regions on a smaller scale,

and are also important capture fisheries species (FAO 2016). However, such aquaculture and capture fishery improvement initiatives can have significant environmental impact where fish escape and establish local populations. Many species of *Oreochromis* have a substantial invasive potential, exhibiting trophic plasticity that enables broad resource use, and a tendency to hybridise (Genner et al. 2013; Shechonge et al. 2019), and now four species of *Oreochromis* are listed on the Global Invasive Species Index (IUCN 2018). Despite certain species exhibiting strong invasive potential, some species within the the genus are severely range restricted and thus are threatened, with 19 species (50%) categorised as '"Near Threatened to Critically Endangered", of which seven species are listed as Critically Endangered (IUCN Red List, v. 2017/3).

Although the higher-level taxonomic categories of cichlids formerly referred to as "Tilapia" have received recent attention (e.g., Dunz and Schliewen 2013), questions remain regarding the factors facilitating the diversification within *Oreochromis*. However, previous phylogenetic studies included limited taxonomic coverage of *Oreochromis*, and delivered conflicting results. These have been largely based on mtDNA markers, with the exception of Schwarzer et al. (2009), Dunz & Schliewen (2013), and Matschiner *et al.* (2017) that included nuclear loci, but only a maximum of four species of *Oreochromis* for the earlier studies, and seven species for the latter study. A recent study investigating actinopterygian relationships (Rabosky *et al.* 2018), included 17 species of *Oreochromis* and two species of *Alcolapia* for both mitochondrial and nuclear data (expanding slightly on Rabosky *et al.* 2013), and while all taxa in this study included ND2, inclusion of nuclear data was very limited, with only *O. niloticus* and *O. tanganicae* having reasonable coverage.

The soda lake cichlid species in the genus *Alcolapia* were originally described as a member of *Tilapia* and subsequently included by Trewavas (1983) as subspecies within a subgenus of *Oreochromis,* as *O. (Alcolapia) alcalicus alcalicus* and *O. (Alcolapia) alcalicus grahami*. Seegers et al. (1999) elevated the subgenus *Alcolapia* to genus based on mtDNA sequence data, and later revised the species name *alcalicus* to *alcalica* to agree with the feminine genus (Seegers 2008). However, while molecular analyses have consistently resolved a monophyletic *Alcolapia*, that is shown to nest within *Oreochromis*, the specific relationship to *Oreochromis* is unresolved, with various taxa having been hypothesised to be the sister species to the *Alcolapia* clade: *O. amphimelas* (Seegers *et al.* 1999; Nagl *et al.* 2001), *O.*

malagarasi (Seegers *et al.* 1999), *O. tanganicae* (Schwarzer *et al.* 2009, Dunz & Schliewen 2013) and *O. variabilis* (Kavembe *et al.* 2013, Matschiner *et al.* 2017, Rabosky et al. 2018). Other than *O. amphimelas,* the other three species previously suggested as alternative sister taxa to *Alcolapia* (i.e. *O. malagarasi*, *O. tanganicae, O. variabilis*) are not found in soda lake conditions. Based on the lack of a densely sampled phylogeny for *Oreochromis* and *Alcolapia*, we generated a comprehensive phylogeny for the group using multiple markers from the mitochondrial and nuclear genomes with near-complete coverage of sampled loci.

Adaptation to adverse environments

Of the 44 currently recognised species and subspecies of *Oreochromis*, excluding *Alcolapia*, nine are known to tolerate or occur in environments with elevated salinity or temperature, but only *O. amphimelas* and *Alcolapia* have adapted to the high alkalinity and low dissolved oxygen levels found in soda lake conditions (see Table 1). Tolerance to temperature and salinity are intrinsically linked, with lower temperature ranges tolerated in saline conditions than in freshwater in some species of tilapia (reviewed in Philippart & Ruwet 1982), and these two parameters are considered to be the determining factors in the distribution of several species. Several species of *Oreochromis* are euryhaline and acclimatise to a range of levels of salinity (from freshwater through to seawater), including *O. urolepis* and *O. mossambicus* that naturally occur in estuarine conditions and have been widely used in aquaculture due to their salinity tolerance (e.g., Riedel & Costa-Pierce 2005; Sardella & Brauner 2008; Ulotu et al. 2016). The typically freshwater *O. niloticus* (composed of seven subspecies) includes members that have successfully colonised thermal hot springs (Bezault et al. 2007, Ndiwa et al. 2014), while other *Oreochromis* species thrive in the high-pH volcanic springs feeding the soda lakes of East Africa (Trewavas 1983). In particular, *O. amphimelas* occurs in the springs and main water bodies of the seasonal soda-like Lakes Manyara, Eyasi, Sulungali, Kitangiri, and Singida, Tanzania. This species co-occurs with introduced populations of *O. niloticus* in the Sulungali, Kitangiri and Singida lakes and alongside introduced populations of *O. esculentus* in Kitangiri and Singida. The genus *Alcolapia* has diversified in the even more extreme environment of the nearby soda lakes: Lakes Natron in Tanzania and Magadi in Kenya (Seegers et al. 1999, Ford et al. 2015, 2016) and occurs in alkaline hydrothermal

springs feeding the lakes. These hot, hyper saline and highly alkaline soda springs represent some of the most extreme aquatic environments known supporting fish life. It is unclear whether adaptation to soda waters occurred early in the evolution of this lineage or arose more recently; and whether it arose only once or multiple times. Resolving the species relationships of the genera *Oreochromis* and *Alcolapia* will enable these hypotheses to be tested.

Based on our comprehensive multi-marker phylogeny, we examine the taxonomic status of the genus *Alcolapia* to test whether *Alcolapia* and *Oreochromis* are reciprocally monophyletic. We also test for multiple origins of a) tolerance to increased salinity and temperature typical of soda conditions, and b) two male secondary sexual characteristics: extended jaws and the genital tassel (long bifid filaments that develop in the breeding season on the genital papillae), that have historically been used to delimit subgenera of *Oreochromis*.

2. Materials and methods

2.1 Species sampling

A total of 28 species from 33 described species of *Oreochromis,* as well as all four species of *Alcolapia* (using taxonomic data compiled by Eschmeyer et al. 2018), are included in this study. Multiple wild samples of each species, and where possible subspecies, from across Africa were included totalling 105 samples (Appendix A, Table S1). Specimens were collected mainly using seine nets, with specimens euthanised with an overdose of clove oil and subsequently preserved in 70-80% ethanol. Fin-clips or muscle tissue were taken for molecular analysis and were preserved in 96-100% ethanol. While this study endeavoured to produce a comprehensive phylogeny of all species of *Oreochromis*, five species could not be obtained, including *O. aureus* (although common in some countries as a food fish, we only included wild samples with known localities), *O. ismailiaensis*, *O. lidole, O. saka,* and *O. spilurus*. However, of these species we believe *O. lidole* to be functionally extinct based on our personal extensive field observations (GFT, MJG), and it is likely that *O. saka* is a geographic variant (and junior synonym) of *O. karongae* (Turner & Robinson 1991). *Oreochromis ismailiaensis* has also not been seen since the original description and the type locality is reported to have been converted to a concrete channel devoid of fish life (A. Dunz pers. comm.), so this too may be extinct. As such (excluding *O. lidole, O. ismailiaensis* and *O.*

saka as extinct or invalid species), our study represents ~93% of all *Oreochromis* species, and all *Alcolapia* species (94% when *Oreochromis* and *Alcolapia* are combined). We included two *Sarotherodon* species (*S. galilaeus* and *S. mvogoi*) based on their close association to *Oreochromis* and *Alcolapia* (Dunz and Schliewen 2013), and the more distantly related *Coptodon rendalli* as an outgroup taxa. For the mtDNA dataset only, a further 16 samples were included based on ND2 GenBank sequences (Table S1). These included samples referred to as *Oreochromis aureus* (three samples), as well as coverage of other Oreochromine genera, including five of the ten genera (Dunz and Schliewen 2013), *Iranocichla*, *Konia*, S*arotherodon* (eight further species)*, Stomatepia,* and *Tristramella*, allowing us to investigate the monophyly of *Oreochromis*.

2.2 Molecular markers

As cichlids are known to exhibit mito-nuclear discordance (e.g., Rognon & Guyomard 2003; Seehausen 2003; Schliewen & Klee 2004; Egger et al. 2007, Alter et al. 2017), we selected six nuclear loci to target a presumed rapidly evolving clade based on the age of the oldest member of this tribe (Dunz & Schliewen 2013). These included the recently developed exon-primed intron crossing (EPIC) markers BMP4, CCNG1, GAPDHS, TYR, b2m (Meyer & Salzburger, 2012) and the nuclear intron S7 intron 1 (Chow & Hazama, 1998), which has previously been used in cichlid studies (Schelly et al., 2006; Schwarzer et al., 2009). We also selected two mitochondrial (mtDNA) genes: NADH dehydrogenase 2 (ND2), frequently used in cichlid phylogenetics (e.g. Klett & Meyer, 2002; Day et al., 2007; Schwarzer et al., 2009; Dunz & Schliewen, 2013), and 16S rRNA (e.g. Farias et al., 1999; Schwarzer et al., 2009) to determine if there was discordance between nuclear and mitochondrial datasets. We sequenced all samples where possible (see Appendix A, Table 1).

2.3 DNA Extraction, amplification and sequencing

Genomic DNA was extracted from fin clip samples or muscle tissue stored in 95% ethanol using the Qiagen DNeasy Blood and Tissue kit. The molecular markers were amplified using the Polymerase Chain Reaction. 1µl of the DNA extraction was added to 12.5µl of MyTaq™ Mix (Bioline, UK), 9.5µl of water, and 1µl each of the 10M forward and reverse primers (Sigma-Aldrich, UK), to give a 25µl total reaction

194 volume. The primers and reaction conditions for each gene are shown in Appendix A, Table S2. Cleaned
195 PCR products were sequenced on a 3730xl DNA Analyser (Applied Biosystems).
196
197 2.4 Alignment and partitioning scheme
198 Sequence data was edited using Sequencher 5.4 (Gene Codes Corporation, Ann Arbor, MI USA) and
199 GENEIOUS v. 6.0.6 (Biomatters) (Kearse et al. 2012) in which contigs were assembled from the forward
200 and reverse sequences. Sequences were subsequently aligned using MUSCLE in GENEIOUS (Kearse et
201 al. 2012) using default parameters, with alignments checked for stop codons and reading frame shifts.
202 The concatenated nuclear dataset (101 samples) included a total of 3092 bp: BMP4 (482 bp), CCNG1
203 (650 bp), GAPDHS (436 bp), TYR (561 bp), b2m (482 bp) and S7 (481 bp). We, however, removed a
204 hyper-variable region (42 bp) from exon 2 of the b2m loci (from 144 and 185 bp) as it was shown to
205 contain two dominant haplotypes and a high density (14) of variable sites. As the possible causes of
206 patterns like this include strong selection, introgression from more divergent species, or paralogous
207 sequences (although our sequences of b2m aligned against a previous dataset for this region [Meyer &
208 Salzburger, 2012]), they would likely erroneously influence phylogenetic reconstruction. The final
209 dataset for the nuclear data was therefore 3050 bp. The mitochondrial dataset (116 samples) included
210 a total of 1582 bp: ND2 (1047 bp) and 16S (535 bp), with each dataset analysed separately.

211 The optimal partitioning scheme and model choices were assessed with PartitionFinder v.2.1.1 (Lanfear
212 et al., 2017) using the greedy algorithm (Huelsenbeck and Ronquist, 2001) and assessed using the
213 Bayesian Information Criteria (BIC). For the nuclear concatenated analyses we defined two subsets for
214 the nuclear (nuDNA) dataset (combining introns vs. exons) and four subsets for the mitochondrial
215 (mtDNA) dataset (each codon position for ND2, plus 16S). For the species tree analyses it was more
216 appropriate to treat the six nuclear loci as separate genes (as opposed to the exon vs. intron partitions
217 in the concatenated analysis), as the most important factor in species tree analysis is variation in the
218 gene tree, as genes will (more or less) have different histories. We also checked to see if implementing
219 the partitions by loci made any difference to the outcome of the nuclear concatenated analysis. To obtain
220 evolutionary models for these partitions we re-ran PartitionFinder. Resulting models (see Table 2) were
221 implemented in subsequent phylogenetic analyses.

2.5 Phylogenetic inference

Phylogenetic analyses were run on 1) the concatenated nuDNA dataset, and 2) the concatenated mtDNA data. Each dataset was analysed using Bayesian Phylogenetic Inference (BI) and Maximum Likelihood (ML), and the nuDNA dataset was also analysed using a Bayesian multispecies coalescent approach. Bayesian Phylogenetic Inference was implemented using MrBayes v.3.2.6 (Ronquist et al. 2012) in which analyses were run for 50,000,000 generations using four Markov chains (three heated, one cold, heating parameter 0.4) with default priors, implementing the models as defined by PartitionFinder. Maximum Likelihood analyses were run using GARLI v.2.01 (Zwickl 2006), again implementing the models defined by PartitionFinder, and running 100 bootstrap (BS) replicates. All analyses were run on CIPRES Science Gateway server (Miller et al., 2010). The convergence of MCMC runs and burn-in were assessed in Tracer v.1.7 (Rambaut et al., 2018) and FigTree v.1.4 (Rambaut, 2009) was used to visualise trees.

2.6 Non-parametric likelihood-based tests

The alternative phylogenetic topologies generated from the mt- and nuDNA datasets were evaluated using the approximately unbiased (AU) test of Shimodaira (2002), who considers this test more accurate than the Shimodaira-Hasegawa (SH) test. ML trees for each dataset were generated in Garli v.2.01 (Zwickl 2006) using matrices containing the same one sample of each ingroup and outgroup species. These trees were imported into PAUP v.4.0b 10 (Swofford, 2002) and site likelihood scores generated. The resulting site likelihood scores were imported and run in the program CONSEL v0.20 (Shimodaira and Hasegawa, 2001).

2.7 Calibration selection and species trees analyses

Although our study was not focused on investigating divergence dates, generation of a species tree to investigate species relationships and trait evolution required the selection of calibrations. The fossil

record of the Oreochromini is represented by several fossils. The oldest of these, †*Sarotherodon martyni* (Van Couvering, 1982) from the Ngorora Formation, Lake Turkana, Kenya (late Miocene: 12.0–9.3 Ma), is considered to belong to *Oreochromis* (Murray and Stewart 1999), although this affinity has been debated (Carnevale et al. 2003). A recent discovery of eight well preserved fossil skeletons from the nearby site, Kabchore, which is middle Miocene (12.5 Ma) are attributed to *Oreochromis* based on unique character combinations and meristic traits (Penk et al. 2018). Based on these fossils (and the occurrence of †*Sarotherodon martyni*), the older age is used to constrain *Oreochromis* and is preferred here to †*Oreochromis lorenzoi* (Carnevale et al. 2003) from the upper Miocene Messinian (7.246 Ma and 5.333 Ma), which has previously been applied as a constraint (Schwarzer et al. 2009). As the fossil record represents a minimum age, we also used a secondary calibration from Rabosky et al. (2018), which is based on a large teleost wide dataset and calibrated with a larger number of fossils, to provide a maximum age (14.71 Ma) for this node.

The Bayesian multispecies coalescent (MSC) method was applied to our nuclear data because it accounts for the coalescent process and therefore accommodates ILS (Flouri et al. 2018). Since our study is focused on closely related young species we removed the outgroup species *Coptodon* spp. to reduce rate heterogeneity. We initially used the program *BEAST v2.4.8 (Bouckaert et al. 2014). Clock models were unlinked across loci, in which a log-normal relaxed clock was selected for the S7 and GAPDHS loci, while a strict clock was selected for all other loci based on the ucldStdev values (which were <1) from an initial run. Site models were implemented based on the results of PartitionFinder in which the nuDNA data was partitioned according to loci (see section 2.4) for these analyses. Analyses were run using the Birth-Death (BD) Model. We applied the maximum and minimum calibrations described above using a uniform prior, selecting 'use originate' = true. Population sizes in the multi-species coalescent were modelled using the 'piecewise linear and constant root' setting.

A further *BEAST analysis of all loci (nuclear and mitochondrial DNA) was also performed as this is required for running JML software (see section 2.8). All settings were the same as described for the nuclear tree species analysis. The mtDNA data was treated as a single locus (see Table 2) in which a strict clock was preferred as indicated by the ucldStdev value which was <1. For each dataset (i.e.

nuDNA, and nuDNA + mtDNA) three analyses were run with chain length 500,000,000, and convergence was checked in Tracer v.1.7, as for the MrBayes analysis. Most ESS values for the nuDNA *BEAST analyses were >200, with treePriors and clock rates >100, but some values (posterior, species coalescent and popMean) were between 96-93. All ESS values for the nuDNA + mtDNA species tree were >200. Runs were combined using TreeAnnotator, and resampled, discarding 25% burnin, and visualised using FigTree v.1.4.3.

We also used the program Bayesian Phylogenetics and Phylogeography (BPP) v 4.0 (Flouri et al. 2018) (method A01) and performed runs of 30,000,000 MCMC iterations testing alternative priors. The calibrations previously described were also applied to this analysis. Although we did not obtain convergence across the runs on the maximum a posteriori tree (MAP) we obtained convergence on the majority rule consensus (MRC) tree, which was identical across all runs. We tried two priors on the root age (tau prior) 1) Inv-Gamma (3,0.002), and 2) Inv-Gamma (2,0.05), and both priors yielded the same (MRC) trees.

2.8 Testing for hybridisation

Due to discordance between mitochondrial and nuclear trees (see Results 3.1) we attempted to investigate if we could distinguish between introgression and incomplete lineage sorting (ILS) using the method described by Joly et al. (2009), implemented in JML v.1.3.1 (Joly 2012). This method uses posterior predictive checking by comparing the minimum sequence distance between two species to test if the minimum distance is smaller than expected under a regime not accounting for hybridisation. However, we acknowledge that our dataset does not include many samples per species, and that loci from our nuclear dataset are short, and therefore lack power of longer sequences (see Joly et al. 2009). As this method uses the posterior distributions of species trees, population sizes and divergence times, we generated a further coalescent tree from all loci (see section 2.7), ensuring that all loci were unlinked to obtain mutation rates needed for JML. JModelTest v.2.1.10 (Guindon and Gascuel, 2003; Darriba et al. 2012) was implemented to obtain models for individual loci and also to obtain settings for the .jml.ctl

file of JML (state frequencies, transition-transversion ratio, proportion of invariable sites, gamma rate heterogeneity). We ran 1000 simulations for each of the three selected loci, mtDNA (locus rate = 0.0038), BMP4 (locus rate = 0.00036) and TYR (locus rate = 0.000656) pairwise distance comparisons. A Benjamini-Hochberg correction was applied to the results using R v.3.5 (R Core Team, 2018).

2.9 Trait analyses

We tested for correlated evolution of traits using BayesTraits v3.0.1 (Pagel et al. 2004; Pagel & Meade 2006), which was also used for ancestral state reconstruction. We focused on trait data for tolerance to soda conditions (salinity, temperature, and pH) with species states derived from the literature (see Table 1). As tolerance to elevated salinity and temperature was most common, we tested for correlated evolution between these traits, and separately reconstructed ancestral states for soda adaptation, defined as comprising all three tolerance traits found in a species: salinity, temperature, and pH. We also examined the correlated evolution of phenotypic secondary sexual male characteristics that have been used in previous taxonomic analysis: the genital tassel, and extended male jaws. Although the two traits have not previously been implicated to have correlated evolution, the two traits are used as diagnostic characters to separate clades and subgenera of *Oreochromis*, and the traits do not co-occur, so we tested whether their presence/absence was correlated. We ran the ancestral state reconstruction analyses on the nuclear datasets only, using both the MrBayes (non-ultrametric) and *BEAST (ultrametric) phylogenies. We did not ultrametricise the MrBayes tree, as branch lengths can have a substantial effect on ancestral state reconstructions (McCann et al. 2016), and following recommendations from Cusimano & Renner (2014) to run reconstructions on more than one type of branch length depiction we therefore include both the phylogram and ultrametric trees. The MrBayes nuclear concatenated tree was pruned to include only one sample per species (or subspecies, where relevant). One sample each was included for subspecies of three species (specifically: *O. niloticus niloticus*; *O. niloticus cancellatus*; *O. niloticus filoa*; and *O. placidus placidus*; *O. placidus ruvumae;* and *O. shiranus shiranus, O. shiranus chilwae*), as plausibly the subspecies may exhibit different adaptations / morphology from each other and so were coded separately. The resulting tree contained 40 tips (including subspecies of *Oreochromis*,

species of *Alcolapia*, and the three outgroup species). Details of the specimens included in the pruned tree are given in Appendix A, Table S3. No pruning was required for the *BEAST species tree as there was only one tip per species (total of 36 tips), which also did not include all the respective subspecies of *O. shiranus* and *O. niloticus* as the availability of only single samples meant that they could not be included in the multispecies coalescent approach. The BayesTraits analysis tested the tolerance (salinity and temperature) and morphological characters separately, using the Discrete analysis mode (testing two binary characters) and comparing the independent model (no correlation of shifts between the two traits) with dependent (correlated shifts) models (see Table S3 for how traits were coded per species). Each analysis (independent and dependent) was run for 5 independent runs, with 10 million MCMC iterations, with the first million discarded as burnin, and the stepping stone sampler set to use 100 stones and run each stone for 10^4 iterations. Tree branch lengths were scaled to a mean of 0.1 using the ScaleTrees command, following the recommendations of the BayesTrait manual. Priors for the rate parameters (the rate coefficient for the gain/loss of each trait respectively) were set based on initial Maximum Likelihood runs in BayesTrait. Based on the ML rates estimates, the MCMC analysis on the MrBayes phylogeny used a hyperprior for all rate coefficients specifying an exponential prior seeded from a uniform distribution on the interval 0 to 10, and those on the *BEAST trees used an exponential prior seeded from a uniform distribution on the interval 0 to 1. Mixing of the chains was checked in the output files to ensure acceptance rates were in the range 20-40%; convergence and ESS were checked using Tracer v1.6. The results for the independent and dependent models based on the marginal likelihood from the stepping stone sampler (expressed on a natural log scale) were compared using Log Bayes Factors calculated as:

Log Bayes Factors = 2(log marginal likelihood complex model - log marginal likelihood simple model)

Interpretation of the values was based on Gilks (1996) as described in the BayesTraits manual, specifically: a log BF factor of <2 is interpreted as weak evidence, >2 as positive evidence, 5-10 as strong evidence, and >10 as very strong evidence.

The tests of correlated evolution suggested that tolerance to salinity and temperature were correlated traits (Results Section 3.3). However, as salinity and thermal tolerance were predominantly

concentrated in one clade (*Alcolapia* and *O. amphimelas*), which could potentially generate a spurious pattern of correlation (Uyeda et al. 2018), we ran two separate ancestral reconstructions coding the two traits either as independent or dependent (correlated) traits. The mean values of the proportional likelihoods for each node was calculated with Excel. We also separately reconstructed a 'soda' adaptation trait for which tip species were coded as present if the species exhibited elevated tolerance to all three parameters (temperature, salinity, and pH). We reconstructed ancestral traits for the phenotypic traits (genital tassel and male jaw morphology) independently, as they did not exhibit evidence of correlated trait evolution (see Results). Input files were prepared for BayesTraits using TreeGraph2 (Stöver and Müller 2010), and results of the ancestral state reconstruction analysis were visualised using ggtree 1.12.14 (Yu et al. 2017) in R v3.5 (R Core Team, 2018). For the analyses using the *BEAST trees, ancestral state reconstructions were conducted on a reduced set of trees (resampled to 25,001 trees in TreeAnnotator), and visualised by plotting on the Maximum Clade Credibility tree.

3. Results

3.1 Phylogenetic relationships

The nuclear species tree (Figure 1), BI (Supplementary Figure S1) and ML concatenated (data not shown, but BS values included on Figure S1) trees were reasonably similar in topology, particularly regarding subclades, although there were several instances of taxa occurring in alternative positions between the concatenated and species trees, most notably the sister group of *Alcolapia* (discussed below). The mtDNA concatenated trees generated using BI and ML were largely congruent (see Figures 1b, Supplementary Figures S2). However, comparison of nu- and mtDNA trees showed high levels of mito-nuclear discordance from the strikingly different placement of certain clades and taxa (Figure 1), and there are instances where different groups appear to be well resolved and monophyletic in trees built from the different sets of loci (Figure 1, Supplementary Figures S1, S2). The AU test based on the ML concatenated trees resulted in the mtDNA tree (-ln L 7024.31) being significantly worse fit to the data (P<0.001) than the nuclear tree (-ln L 6516.82), but we discuss both topologies below. As mitochondrial data is prone to introgression in cichlids, we used the nuclear species trees for

downstream analyses. A multispecies coalescent approach is preferred as it accounts for gene tree-species tree incongruence that arise due to population level processes, and is particularly suitable for more recently diverged groups (e.g. Ogilvie et al. 2016, 2017 and refs therein), although we acknowledge that the nuclear genome may also be introgressed in this group of fishes.

The sister group of Alcolapia

Both the *BEAST (Figure 1a) and BPP (Supplementary Figure S3) versions of the nuDNA species tree resolved *O. esculentus* (a freshwater species) as the sister group to *Alcolapia* (extreme soda-lakes), with *O. amphimelas* (seasonal soda-lakes) as sister to these lineages. This is however not well supported (0.55 / 0.69 respectively), and a preliminary *BEAST analysis including the 42 bp hyper-variable data (removed from subsequent analyses due to high variability) conversely resolved *O. amphimelas* as the sister group (data not shown). The nuDNA concatenated phylogenies (Figure 1b, Appendix A Figure S1) (with or without the 42 bp region) resolved the sister group to the *Alcolapia* clade as *O. amphimelas* (BPP 1/0.90 [concatenated/species tree]; BS 91), with *O. esculentus* resolved (BPP 1/0.90; BS 89) as sister to the *Alcolapia* + *O. amphimelas* group. All these species are closely distributed geographically occurring in NW Tanzania and SW Kenya. The nuDNA concatenated tree (Appendix A Figure S1) shows that there is also some resolution within the *Alcolapia* clade itself, with the geographically isolated *A. grahami* (Lake Magadi, Kenya) resolved (BPP 0.99) and most closely related to the 'northern' *A. alcalica* from Lake Natron, although there is poor resolution regarding the relationships among the three sympatric Lake Natron species ('southern' *A. alcalica*, *A. latilabris*, *A. ndalalani*). In contrast, the mtDNA concatenated phylogeny (Figure 1c, Appendix A Figure S2) placed the *Alcolapia* clade as the sister group to (BPP 0.86/BS 56) a mixed assemblage of six species (including *O. esculentus*), which are not themselves phylogenetically resolved, with *O. amphimelas* resolved as the sister taxon to this clade. There is also no resolution of the relationships among the constituent species of *Alcolapia*. When the ND2 data alone is analysed, *Alcolapia* + *O. amphimelas* are sister taxa, albeit with low support, and the 'mixed assemblage' is the sister group to this clade (data not shown) indicating differing signals from the mtDNA loci.

Relationships of African Great lake taxa

There are also disparities in the placement of some of the African Great Lake taxa. For example, the Lake Tanganyika basin species *O. tanganicae* and *O. malagarasi* are sister taxa in all the nuDNA phylogenies, but are not close relatives in the mtDNA tree. In the mtDNA tree a sister relationship of *O. malagarasi* and *O. upembae* (occurs in the Congo Basin, and East Africa, specifically within the Lake Tanganyika catchment) is resolved, which is in line with Trewavas's (1983) reporting of close relationships between these two species based on phenotypic characters. Other placements that differ between the nuDNA and mtDNA phylogenies included riverine taxa, *O. niloticus* (a wide-ranging taxon whose native range is across the Nilo-Sudan ichthyo-province) which groups with *O. lepidurus* (occurs in the Lower Congo River) and *O. upembae* in the nuDNA tree. Conversely *O. niloticus* groups with *O. angolensis* (Quanza ichthyo-province) and *O. schwebischi* (Lower Guinea Forest ichthyo-province) in the mtDNA tree. In the mtDNA trees subspecies *O. niloticus cancellatus* and *O. niloticus filoa* (Appendix A Figure S2) were consistently resolved as being more closely related to each other than to *O. niloticus niloticus,* but there was poorer resolution of *O. niloticus* subspecies in the nuDNA concatenated tree (Appendix A Figure S1). Notably the Lower Congo River species *O. lepidurus* is closely related to a taxon that occurs in Lake Tanganyika (or its catchment) in either tree: it is the sister taxon to *O. tanganicae* in the mtDNA tree, and sister to the clade comprising *O. upembae* and *O. niloticus* in the nuDNA tree. A connection between these water bodies has been demonstrated in other lineages such as lamprologine cichlids (e.g. Day et al. 2007) and mastacembelid spiny eels (Day et al. 2017).

However, there are areas of some congruence between the trees built from mtDNA versus nuDNA. In particular, species from the Lake Malawi catchment and the formerly connected Ruvuma (also known as the Rovuma) catchment (*O. shiranus, O. squamipinnis, O. karongae, O. chungruruensis, O. placidus ruvumae*) form a well supported clade in both the mtDNA and nuDNA species trees. The concatenated nuclear tree supports a variation of this grouping, with the exception of *O. placidus ruvumae,* which conversely groups within a larger clade that is sister to Lake Malawi catchment species. In both nuclear trees (species and concatenated), *O. placidus* (Zambezi river) is also a member of this group, whereas this taxon groups with other largely southern African species in the mtDNA tree.

435 *Pangani relationships*

436 In both the nuDNA concatenated, and mtDNA trees, *O. jipe* ("pangani") and the type species of the genus,
437 *O. hunteri* (both endemic to the upper Pangani system in the Tanzania/Kenya border region of the East
438 African ichthyo-province) are not monophyletic. This result is also suggested from the mtDNA tree. In
439 this tree *O. jipe* and *O. mweruensis* (from the Zambezi ichthyo-province) form a clade in which
440 constituent species are non-monophyletic, although the *O. jipe* sample ZSM 1065 (not included in the
441 nuDNA analysis) forms a clade with *O. hunteri* and *O. korogwe*, highlighting that mtDNA loci may not
442 always correctly resolve species boundaries. However, when our nuclear data was analysed using
443 species tree methods *O. hunteri* and *O. jipe* are resolved as monophyletic, although with weak support.
444 A recent study focused on *O. hunteri* (Moser et al. 2018) based on mtDNA control region (830 bp) also
445 supported a close relationship between *O. jipe* and *O. hunteri*, but this study only included a small subset
446 of *Oreochromis* species.

447

448 *Oreochromis monophyly*

449 The inclusion of samples from GenBank within the mtDNA analysis (see Supplementary Figures S2)
450 allowed us to test the monophyly of *Oreochromis*. With the exception of sequences uploaded as *O. aureus*
451 (for which we did not have access to voucher specimens), *Oreochromis* was resolved as monophyletic,
452 or rather as paraphyletic with *Alcolapia* nested within it. The position of *O. aureus* (a Nilo-Sudanic
453 species) grouped within the Lake Barombi Mbo radiation, and outside of *Oreochromis* likely implies that
454 the sampled taxa are hybrids or were originally mis-identified. However, although *Oreochromis aureus*
455 has not been included in recent molecular studies of tilapiine relationships (e.g. Schwarzer et al 2009;
456 Dunz and Schliewen 2013), an allozyme study previously resolved *O. aureus* at the base of the
457 *Oreochromis* clade (Pouyaud and Agnèse 1995). Of the four GenBank *O. aureus* samples used to sequence
458 NADH, only one is in a published paper (DQ465029.1; Cnaani et al. 2008), and was collected from stocks
459 of *O. aureus* in Israel that were originally sourced from Lakes Hula (Israel) and Manzala (Egypt), both of
460 which are also inhabited by *S. galilaeus*. Trewavas (1983) notes several characters that distinguish *S.*
461 *galilaeus* from co-occurring *O. aureus* and *O. niloticus*, including the depth of the pre-orbital bone.

462 Intergeneric hybrids between *Oreochromis* and *Sarotherodon* are viable and have been produced in
463 aquaculture strain development (using *in vitro* fertilisation; Bezault et al. 2012), but we are not aware
464 of any reports of intergeneric crosses in the wild, especially in natural sympatry zones. Further samples
465 of *O. aureus* are required to investigate this placement fully and ensure that *S. galilaeus* samples have
466 not been mis-identified as *O. aureus*.

467

468

469 3.2 Assessment of hybridisation

470 Results from the JML analysis revealed no support for introgression, with no significant signal of
471 hybridisation after applying the Benjamini-Hochberg correction in any of the loci tested (mtDNA, TYR,
472 BMP4). These results suggest that incomplete lineage sorting could explain the incongruence in mtDNA
473 and nuclear datasets (rather than a signal introgression). However, the large number of pairwise
474 comparisons (630) mean that testing across the entire phylogeny is unlikely to uncover signals of
475 hybridisation, and future analyses may focus on specific pairwise comparisons of interest. We suggest
476 that additional data would need to be included to help refine this analysis, but that ultimately
477 examination of species of *Oreochromis* using genome-wide data would provide a powerful approach to
478 test hybridisation hypotheses.

479

480 3.3 Diversification and ancestral state reconstruction

481 The BayesTraits analysis for environmental tolerance traits (salinity and thermal tolerance) using the
482 Discrete model for the nuclear *BEAST species tree gave a marginal log likelihood for the independent
483 model of -26.50 (with a standard deviation of 0.04 across 5 runs), while the dependent model had a
484 marginal log likelihood of -24.72 (s.d. 0.02). These resulted in a log Bayes Factor of 3.56, indicating
485 moderate support for the dependent model, suggesting that the shifts in adaptation to salinity and
486 thermal tolerance may be correlated. Results when using the nuclear concatenated MrBayes tree were
487 more conclusive, with the 5 runs resulting in a log Bayes Factor of 6.78, indicating strong support for
488 correlation of the traits. For the morphological traits (genital tassel and extended male jaw morphology)
489 using the nuclear *BEAST species tree the independent model marginal log likelihood was -39.82 (s.d.

0.06) while that for the dependent model was -39.18 (s.d. 0.04). The log BF was 1.30, indicating that there was no support for the complex model (dependence of trait shifts), and suggesting that the two phenotypic traits do not exhibit correlated rate shifts. Results when using the nuclear concatenated MrBayes tree were similar, with the 5 runs resulting in a log Bayes Factor of -0.22, indicating no support for correlation of the phenotypic traits.

The ancestral state reconstruction indicated that adaptation to increased salinity and/or temperature has occurred multiple times within the genus *Oreochromis*, but that adaptation to soda lake conditions has likely occurred once, in the lineage leading to *Alcolapia* + *O. esculentus* + *O. amphimelas* (Figure 2A). The results were similar (and more conclusive) in the analysis using the concatenated nuclear phylogeny (Figure S4A), where soda adaptation is likely to have occurred only once in the lineage leading to *Alcolapia* + *O. amphimelas*. Reconstructing thermal and salinity tolerance independently also showed that adaptation is likely to have occurred multiple times throughout the phylogeny (Figure S5). The reconstruction of the phenotypic traits (Figure 2B) indicated that the secondary sexual characteristics or extended jaw and genital tassel are not correlated, and that both are likely to have evolved multiple times, suggesting that they are not useful taxonomic diagnostic characteristics for this genus. The reconstruction of phenotypic characters on the MrBayes phylogeny gave similar results to that using the *BEAST phylogeny, and suggested that the morphological traits evolved independently in multiple clades (Figure S4B).

4. Discussion

Here, we present the first comprehensive phylogenetic analysis of the cichlid genus *Oreochromis* using multi-marker molecular datasets comprising nuclear and mitochondrial loci, and reveal high levels of incongruence between them. This incongruence could either represent incomplete lineage sorting and/or introgression, and while we did not find a signal for the latter mechanism it cannot be ruled out. Incongruence as a result of these mechanisms is commonly reported in other cichlid studies (e.g., Genner & Turner 2012; Willis et al. 2013; Meier et al. 2017). We suggest that the nuDNA phylogeny is likely to be a better estimate of the species tree, although we acknowledge that additional data would help to resolve conflicting relationships. Based on the nuDNA trees, we show that adaptation to adverse

environmental conditions (i.e. increased salinity and temperature) has occurred multiple times, but that adaptation to extreme (soda) conditions is likely to have occurred once. Irrespective of the dataset (nuDNA or mtDNA), we demonstrate that the *Alcolapia* clade is consistently resolved within the genus *Oreochromis*.

4.1 Taxonomy of the genus *Oreochromis:* effects of mito-nuclear discordance

We observed substantial mito-nuclear discordance in the phylogenetic analysis (Figure 1). We note that several of the relationships in the nuclear analysis are concordant with previous phenotypic classifications based on phenotypic features, but that certain relationships within the mtDNA analysis are also plausible based on phenotypic and geographical range data. The results suggest reticulate evolution could have played a role in the existing genetic relationships, and could be a result of the fact that many tilapiine species are known to have been widely translocated for stocking and aquaculture purposes, although we do not find significant evidence of introgression using JML analysis. Alternative explanations include incomplete lineage sorting that is widely reported in cichlids (e.g., Genner & Turner 2012; Meier et al. 2017; Meyer et al. 2017). Previous analysis of mito-nuclear discordance in the tribe Oreochromini suggested that ancient hybridisation was the more probable explanation of this discordance (Dunz & Schliewen 2013).

The translocation and establishment of non-native species (*O. leucostictus, O. niloticus*) within Tanzania has been recently documented (Shechonge et al. 2018). We note two additional non-native species occurrences in Tanzania from our sampling: *O. leucostictus* (AF042-04) sampled from fish ponds in the Lake Eyasi basin (reportedly stocked from streams surrounding Lake Eyasi), and *O. urolepis* (AF014-01) sampled from irrigation canals draining into Lake Manyara. Male specimens collected from the latter site also exhibited the distinctive extended jaw morphology of sexually mature male *O. urolepis*. To our knowledge, this is the first report of *O. urolepis* sampled in a natural water body outside of its native catchments, that includes the Wami, Ruvu and Rufiji basins. However, we are aware of aquaculture centres rearing *O. urolepis* in Tanga (Mmochi 2017).

We also find taxonomic discrepancy in the resolution of *O. placidus* samples from different geographic areas. Trewavas and Teugels (1991) synonymised *O. placidus placidus* and *O. placidus ruvumae*, but other researchers have reported substantial morphological differences between *O. placidus* specimens from the type locality (Buzi River) and those found in the Ruvuma River (Bills 2004). Our data support this latter finding as in both the nu- and mtDNA phylogenies, *O. placidus ruvumae* samples form a distinct clade from *O. placidus placidus*. However, we find that within the mtDNA tree and nuDNA species tree *O. placidus ruvumae* is either sister or groups within the Lake Malawi catchment clade, whereas in the concatenated nuDNA tree is it more distantly related. The close relationship supported in several of these trees is consistent with recent suggestions that the Ruvuma River was formerly the outflow of Lake Malawi (Ivory *et al.* 2016), supported by the shared presence of *O. shiranus* in the Lake Malawi catchment, in Lake Chiuta (Ruvuma catchment) and Lake Chilwa (endorheic but formerly connected to Ruvuma) (Trewavas 1983). However, the mitochondrial and nuclear trees disagree with morphological hypotheses (Trewavas 1983), although these are not based on cladistic analyses, which placed *O. squamipinnis, O. karongae* and *O. chungruruensis* with the other tasseled species in the subgenus *Nyasalapia,* but grouped *O. shiranus* and *O. placidus* with other species from East coast rivers showing enlarged male jaws in a division of subgenus *Oreochromis*.

The molecular phylogenies and ancestral state reconstruction (Figure 2B) suggest that the male secondary sexual phenotypic characteristics previously used to group species do not represent phylogenetically conserved characters. Specifically, the presence of a genital tassel in males, a defining character of the *Nyasalapia* subgenus rank erected by Thys (1968), does not reliably distinguish clades resolved in our molecular phylogeny, and is likely to have evolved multiple times. Trewavas (1983) reported that other than genital tassel, there were no defining phenotypic characteristics distinguishing *Nyasalapia* from the rest of *Oreochromis* and suggested that the subgeneric value of the tassel was open to question.

Alcolapia is consistently resolved within *Oreochromis* irrespective of dataset and forms a strongly supported clade with *O. amphimelas* and *O. esculentus* irrespective of nuclear analyses performed. All previous phylogenetic studies including the two genera have also resolved *Alcolapia* within *Oreochromis*, albeit with less comprehensive sampling of either genus (Seegers *et al.* 1999; Nagl *et al.*

2001, Schwarzer *et al.* 2009, Dunz & Schliewen 2013, Kavembe *et al.* 2013, Matschiner *et al.* 2017, Rabosky et al. 2018). However, the sister group of *Alcolapia* is contentious, as *O. amphimelas* is strongly supported as its sister group based on concatenated nuclear analyses, whereas, species tree analyses placed *O. esculentus* as sister, albeit with weak support. A species tree analysis of the nuclear data without removing the hyper-variable 42 bp (data not shown) did support the *Alcolapia* + *O. amphimelas* relationship, and it is likely that the uncertainty in the species tree may be attributed to a lack of synapomorphies.

4.2 Systematics Account: Revised classification of *Alcolapia*

Based on the results of our phylogenetic analyses, we propose a revised classification of *Alcolapia*. Given the position of *Alcolapia* within the comprehensively sampled molecular phylogenies presented here, and concordant with previous molecular work, we propose that *Alcolapia* is synonymised with the genus *Oreochromis*, retaining the subgeneric allocation of *Alcolapia*. We recognise *Alcolapia* Thys van den Audenaerde, 1969 as a subgenus within *Oreochromis* Günther, 1889. The synonymy is necessary to reflect a monophyletic taxonomy; *Oreochromis* is paraphyletic unless *Alcolapia* is subsumed within it. While both Trewavas (1983) and Seegers & Tichy (1999) noted several morphological characters for the diagnosis of *Alcolapia* as a sub-genus, most of the characters were shared with *O. amphimelas* and several overlapped with other *Oreochromis* species. The elevation of *Alcolapia* to genus rank was ultimately based on molecular (mtDNA) data (Seegers et al. 1999). However, this is not supported by subsequent molecular analyses, including the present study. As such, the revised classification we propose here follows the previous taxonomy of Seegers & Tichy (1999), namely: *Oreochromis (Alcolapia) alcalicus*, *Oreochromis (Alcolapia) grahami*, *Oreochromis (Alcolapia) latilabris*, and *Oreochromis (Alcolapia) ndalalani*.

An alternative solution to the synonymisation would be to split *Oreochromis* into several genera representing the constituent reciprocally monophyletic groups; however, given the differences in groups between datasets, the current data would not support this taxonomic treatment. In addition to the molecular evidence for synonymising these genera, we note the phenotypic similarities between the species of *Oreochromis* (*Alcolapia)* and *O. amphimelas* reported by Trewavas (1983).

600

601 4.3 Timing and colonisation of extreme conditions

602 Our study shows that there has been repeated adaptation to elevated salinity and temperature
603 throughout the evolutionary history of *Oreochromis* irrespective of phylogenetic hypothesis, but that
604 adaptation to soda conditions (high temperature, salinity, alkalinity, and low dissolved oxygen) has
605 likely occurred once. The placement of *O. esculentus* in a clade with *Alcolapia* and *O. amphimelas* in the
606 species tree analyses (Figure 1A, Supplementary S3), raises the possibility that the adaptation to soda
607 conditions was also gained in *O. esculentus* (Figure 2A), although it does not currently inhabit soda
608 conditions (Table 1). While *O. esculentus* is native to the freshwater Lake Victoria (Njiru et al, 2005;
609 Hallerman and Hilsdorf, 2014), our samples are from Lake Rukwa, a relatively shallow saline lake, where
610 they have been introduced from Lake Victoria (Seegers, 1996). Lake Rukwa became saline around 5,500
611 years ago (Barker et al, 2002), and although conditions (pH 9.19-9.26; temperature 27-32°C; salinity:
612 6,000 mg/L, Haberyan, 1987; Bathymetric survey report, 2014) are not as extreme as the springs
613 inhabited by *Oreochromis (Alcolapia)* (e.g. pH 8-12, temperature 27-42°C, salinity 34,000 mg/L) or *O.*
614 *amphimelas*, they are much higher than Lake Victoria, for example (pH: 8.2-9, temperature: 23-28
615 [surface water temperature], salinity: 97 mg/L) (vanden Bossche & Bernacsek 1990). Of course, Lake
616 Victoria has experienced phases of desiccation (Johnson et al. 1996) and so it is likely that species such
617 as *O. esculentus* will have encountered periods of higher salinity in the past. This is probably true of
618 many other *Oreochromis* populations and thus it is perhaps unsurprising given that there has been
619 multiple adaptations to these conditions throughout the evolution of this group, indicating a likely
620 shared genetic mechanism allowing fish from this genus to adapt to such conditions.

621 Our age estimates of the diversification of the *Oreochromis (Alcolapia)* adaptive radiation at 1.73
622 Ma (95% HPD: 3.20-0.57) coincide with estimates of the date of basin formation for Lakes Natron and
623 Magadi at 1.7 Ma and the formation of the single palaeolake (Orolonga) preceding Natron and Magadi,
624 around *c.*700 Ka (Eugster 1986). The Lake Magadi species *O. (A.) grahami* is estimated to have diverged
625 from the Lake Natron species *O.* (*A.*) *alcalicus* at 0.70 Ma (95% HPD: 1.55-0.007 Ma), suggesting these
626 taxa may have already diverged prior to the separation of the lakes. However, although these dates are

627 generally well before the time that these lakes are suggested to have separated, during an aridity event
628 dated at *c.*11 ka (Williamson et al. 1993) the upper bound is after this timeframe. It is likely that the
629 nuclear loci selected for this study are too slowly evolving to provide a precise estimate of divergence
630 dates for a potentially recent radiation (Ford et al. 2015). More nuclear loci and broader outgroup
631 sampling including additional fossil calibrations should be considered in future studies to test the
632 accuracy of these dates.

633 It has been suggested that *O. amphimelas* (occurs in Lakes Manyara, Eyasi, Sulungali, Kitangiri
634 and Singida) might be a close relative of *Oreochromis (Alcolapia)* based on adaptation to soda conditions
635 - although *O. amphimelas* experiences less extreme conditions (Trewavas 1983) - supporting the
636 findings presented here. There is also a hydrological connection between the two basins, with
637 groundwater flowing northwards from Manyara to Natron and the lowest border of Manyara (~80m
638 above the current lake level) forming an overspill to Natron (Hillaire-Marcel & Casanova 1987; Bachofer
639 *et al.* 2014). However, the suggestion that the two basins were joined in a palaeolake as recently as 10
640 ka (Holdship 1976) has not been supported by geological evidence (Casanova & Hillaire-Marcel 1992).
641 As for Magadi, fossils indicate that Lake Manyara also was inhabited by a larger freshwater cichlid in a
642 previous palaeolake (Schluter *et al.* 1992). However, unlike Natron-Magadi, Manyara now contains only
643 one native species (*O. amphimelas*).
644

645 ## 5 Conclusions

646 The comprehensive molecular phylogenies of *Oreochromis* presented here represent the first attempt
647 to clarify species relationships for this relatively young and widespread African cichlid genus. Trees
648 reconstructed from nuclear sequence data likely give the best hypothesis of relationships, although we
649 recognise that nuclear genomes may also show introgression (e.g. Nevado et al. 2011). The multispecies
650 coalescent approach is preferred over concatenation methods, as it accounts for gene tree- species tree
651 incongruence that arise due to population level processes and has been shown to be especially suited to
652 the estimation of shallower evolutionary relationships (e.g. Ogilvie et al. 2016, 2017; Flouri et al. 2018),
653 but we acknowledge that we include a limited number of loci for these types of analyses. To clarify
654 relationships and better understand the substantial mito-nuclear discordance that we report, further

work should focus on genomic data e.g. ultraconserved element or reduced representation genome-wide sequencing to resolve potential causes of this incongruence and better clarify the areas of conflict (currently in progress). Genomic data will also enable the likely shared genetic mechanism allowing these fish to adapt to adverse aquatic conditions to be investigated. Our results highlight the importance of establishing species relationships within this genus, which contains several commercially important, and many endangered, species.

Funding

We acknowledge the Systematics Research Fund, the Genetics Society Heredity Fieldwork grant, and UCL Graduate scholarship (AGPF); BBSRC/NERC SYNTAX award (JJD); Royal Society Leverhulme Trust Africa Awards (MJG, GFT, BPN).

Acknowledgements

We thank Simon Joly and Andrew Meade for advice in the use of the JML and BayesTraits software programs, respectively. We also thank Wendy Hart for sequencing support, and Etienne Bezault, Emmanuel Vreven, and Julia Schwarzer for providing additional samples. The associate editor Guillermo Orti and reviewers, Michael Matschiner and Prosanta Chakrabarty, provided helpful comments on an earlier version of this manuscript.

Appendix A. Supplementary material

Table S1. Specimen list and GPS coordinates of samples included in the present study, including GenBank accession numbers for molecular sequences used in phylogenetic analysis.

Table S2. Primers and reaction conditions for each gene.

Table S3. Trait coding (adaptation to high temperature, high salinity and high pH, and phenotypic traits) for each species, as implemented in the BayesTraits analysis.

Figure S1. Bayesian phylogeny of *Oreochromis* based on concatenated nuclear data (3092 bp) including 101 samples (*Coptodon* sp. samples [outgroup] are removed from the figure). Support values shown

682 below nodes are Bayesian Posterior Probabilities (BPP). Those above the nodes are bootstrap (BS)
683 values generated using Maximum Likelihood.
684 Figure S2. Bayesian phylogeny of *Oreochromis* based on concatenated mitochondrial data (1582 bp)
685 including 116 samples, including additional sampling of other Oreochromini taxa (*Coptodon* spp.
686 samples [outgroup] are removed from the figure). Support values shown below nodes are Bayesian
687 Posterior Probabilities (BPP). Those above the nodes are bootstrap (BS) values generated using
688 Maximum Likelihood.
689 Figure S3. Species tree based on nuclear data and generated using BPP. Support values are given below
690 branches.
691 Figure S4. Ancestral state reconstruction from BayesTraits analysis based on the MrBayes nuclear
692 concatenated phylogeny. Details, abbreviations and colour coding are as per Figure 2 in the main paper.
693 A) Ancestral state reconstruction of thermal/salinity tolerance (TS) and soda adaptation (So). Pie charts
694 at internal nodes indicate probability of presence/absence of ancestor exhibiting adaptation to soda
695 conditions, from BayesTraits analysis. (pie above node: temperature/salinity tolerance ancestral state
696 reconstruction; pie below node: soda adaptation ancestral state reconstruction). B) Ancestral state
697 reconstruction of phenotypic male secondary sexual characteristics: genital tassel (GT) and extended
698 jaw morphology (EJ). (pie above node: extended jaw morphology ancestral state reconstruction; pie
699 below node: genital tassel ancestral state reconstruction).
700 Figure S5. Ancestral state reconstruction of thermal and salinity tolerance as independent traits. A)
701 Reconstruction using the *BEAST species trees; B) reconstruction using the MrBayes concatenated
702 nuclear phylogeny. (pie above node: thermal tolerance ancestral state reconstruction; pie below node:
703 salinity tolerance ancestral state reconstruction).
704

705 ## References

706 Alter AE, Munshi-South J, Stiassny MLJ (2017) Genome wide SNP data reveal cryptic phylogeographic
707 structure and microallopatric divergence in a rapids-adapted clade of cichlids from the Congo River.
708 *Molecular Ecology*, 26, 1401-1419.

709 Bachofer F, Quénéhervé G, Märker M (2014) The delineation of paleo-shorelines in the Lake Manyara
710 basin using TerraSAR-X data. *Remote Sensing*, 6, 2195-2212.
711 Barker P, Telford R, Gasse F, Thevenon F (2002) Late Pleistocene and Holocene palaeohydrology of Lake
712 Rukwa, Tanzania, inferred from diatom analysis. *Palaeogeography, Palaeoclimatology,*
713 *Palaeoecology*, 187, 295–305.
714 Bezault E, Feder C, Derivaz M *et al*. (2007) Sex determination and temperature-induced sex
715 differentiation in three natural populations of Nile tilapia (*Oreochromis niloticus*) adapted to
716 extreme temperature conditions. *Aquaculture*, 272, S3–S16.
717 Bezault E, Rognon X, Clota F, Gharbi K, Baroiller J-F, Chevassus B (2012) Analysis of the meiotic
718 segregation in intergeneric hybrids of tilapias. *International Journal of Evolutionary Biology*, 2012,
719 817562.
720 Bills IR (2004) A survey of the fishes and fisheries in the Niassa Reserve, Niassa and Cabo Delgado
721 Provinces, Mozambique (10-30/8/2003). SAIAB Investigational Report 69.
722 Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ
723 (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational*
724 *Biology*, 10, e1003537.
725 Carnevale G, Sorbini C, Landini W (2003) *Oreochromis lorenzoi*, a new species of tilapiine cichlid from
726 the Late Miocene of Central Italy. *Journal of Vertebrate Paleontology*, 23, 508–516.
727 Casanova J, Hillaire-Marcel C (1992) Chronology and paleohydrology of Late Quaternary high lake levels
728 in the Manyara basin (Tanzania) from isotopic data (^{18}O, ^{13}C, ^{14}C, Th/U) on fossil stromatolites.
729 *Quaternary Research*, 38, 205–226.
730 Chow S and Hazama K (1998) Universal PCR primers for S7 ribosomal protein gene introns in fish.
731 *Molecular Ecology*, 7, 1255-1256.
732 Cnaani A, Lee BY, Zilberman N, Ozouf-Costaz C, Hulata G, et al (2008) Genetics of sex determination in
733 tilapiine species. *Sex Dev*, 2, 43–54.
734 Cusimano N and Renner SS (2014) Ultrametric trees or phylograms for ancestral state reconstruction -
735 Does it matter? *Taxon,* 36: 721–726.

736 Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and
737 parallel computing. *Nature Methods*, 9(8), 772.
738 Day JJ, Santini S, Garcia-Moreno J (2007) Phylogenetic relationships of the Lake Tanganyika cichlid tribe
739 Lamprologini: The story from mitochondrial DNA. *Molecular Phylogenetics and Evolution*, 45, 629-
740 642.
741 Day JJ, Fages A, Brown KJ, Vreven EJ, Stiassny MLJ, Bills R, Friel JP, Rüber L (2017) Multiple independent
742 colonizations into the Congo Basin during the continental radiation of African *Mastacembelus* spiny-
743 eels. *Journal of Biogeography,* 44, 2308-2318.
744 Dunz AR, Schliewen UK (2013) Molecular phylogeny and revised classification of the haplotilapiine
745 cichlid fishes formerly referred to as "Tilapia." *Molecular Phylogenetics and Evolution*, 68, 64–80.
746 Edgar RC (2004) MUSCLE: A multiple sequence alignment method with reduced time and space
747 complexity. *BMC Bioinformatics*, 19, 1–19.
748 Egger B, Koblmüller S, Sturmbauer C, Sefc KM (2007) Nuclear and mitochondrial data reveal different
749 evolutionary processes in the Lake Tanganyika cichlid genus *Tropheus*. *BMC Evolutionary Biology*,
750 7, 137.
751 Eschmeyer WN (2018) *Catalog of fishes electronic version. Updated 2018*. California Academy of Sciences.
752 FAO. 2016. The State of World Fisheries and Aquaculture 2016. Contributing to food security and
753 nutrition for all. Rome.
754 Farias IP, Ortí G, Sampaio I, Schneider H, Meyer A (1999) Mitochondrial DNA phylogeny of the family
755 Cichlidae: monophyly and fast molecular evolution of the Neotropical assemblage. *Journal of*
756 *Molecular Evolution*, 48, 703–711.
757 Ford AGP, Dasmahapatra KK, Rüber L, Gharbi K, Day JJ (2015) High levels of interspecific gene flow in
758 an endemic cichlid fish adaptive radiation from an extreme lake environment. *Molecular Ecology,*
759 24, 3421–3440.
760 Ford AGP, Rüber L, Newton J, Dasmahapatra KK, Balarin JD, Bruun K, Day JJ (2016) Niche divergence
761 facilitated by fine-scale ecological partitioning in a recent cichlid fish adaptive radiation. *Evolution*,
762 70, 2718–2735.

763 Flouri T, Jiao X, Rannala B, Yang Z (2018) Species tree inference with BPP using genomic sequences and
764 the multispecies coalescent. *Molecular Biology and Evolution*, 35, 2585–2593.
765 Guindon S and Gascuel O (2003). A simple, fast and accurate method to estimate large phylogenies by
766 maximum-likelihood. *Systematic Biology*, 52, 696-704.
767 Genner MJ, Seehausen O, Lunt DH, Joyce DA, Shaw PW, Carvalho GR, Turner GF (2007) Age of cichlids:
768 new dates for ancient lake fish radiations. *Molecular Biology and Evolution*, 24, 1269-1282.
769 Genner MJ, Turner GF (2012) Ancient hybridization and phenotypic novelty within Lake Malawi's
770 cichlid fish radiation. *Molecular Biology and Evolution*, 29, 195–206.
771 Genner MJ, Connell E, Shechonge A, Smith A, Swanstrom J, Mzighani S, Mwijage A, Ngatunga BP, Turner
772 GF (2013) Nile tilapia invades the Lake Malawi catchment. *African Journal of Aquatic Science,* 38
773 (Supplement 1), 85–90.
774 Gilks WR (1996). *Introducing Markov chain Monte Carlo. Markov chain Monte Carlo in practice*. Springer.
775 Hall BG (2011) *Phylogenetic Trees Made Easy: A How-To Manual*. Sinauer Associates, Sunderland, MA.
776 Hallerman E, Hilsdorf AWS (2014) Conservation genetics of tilapias: Seeking to define appropriate units
777 for management. *The Israeli Journal of Aquaculture*, 2014, 1–18.
778 Hillaire-Marcel C, Casanova J (1987) Isotopic hydrology and paleohydrology of the Magadi (Kenya) -
779 Natron (Tanzania) basin during the Late Quaternary. *Palaeogeography, Palaeoclimatology,*
780 *Palaeoecology*, 58, 155-181.
781 IUCN (2018) Global invasive species database. Available at: http://www.iucngisd.org/gisd/. Accessed
782 April 2018.
783 The IUCN Red List of Threatened Species. Version 2017-3. <www.iucnredlist.org>. Accessed April 2018.
784 Ivory SJ, Blome MW, King JW, McGlue MM, Cole JE & Cohen AS (2016) Environmental change explains
785 cichlid adaptive radiation at Lake Malawi over the past 1.2 million years. *Proceedings of the National*
786 *Academy of Sciences of the United States of America*, 113, 11895-11900.
787 Johnson TC, Scholz CA, Talbot MR, Kelts K, Ricketts RD, Ngobi G, Beuning K, Ssemmanda I, McGill JW
788 (1996) Late Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. Science,
789 273, 1091-1093.
790 Joly S (2012) jml: testing hybridization from species trees. *Molecular Ecology Resources*, 12, 179–184.

791 Joly S, McLenachan PA, Lockhart PJ (2009) A statistical approach for distinguishing hybridization and
792 incomplete lineage sorting. *The American Naturalist*, 174, e54-e70.
793 Kavembe GD, Machado-Schiaffino G, Meyer A (2013) Pronounced genetic differentiation of small,
794 isolated and fragmented tilapia populations inhabiting the Magadi Soda Lake in Kenya.
795 *Hydrobiologia*, 739, 55–71.
796 Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S,
797 Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and
798 extendable desktop software platform for the organization and analysis of sequence data.
799 *Bioinformatics*, *28*, 1647–1649.
800 Klett V, Meyer A (2002) What, if anything, is a Tilapia? - Mitochondrial ND2 phylogeny of tilapiines and
801 the evolution of parental care systems in the African cichlid fishes. *Molecular Biology and Evolution*,
802 19, 865–883.
803 Kobayashi M, Msangi S, Batka M, Vannuccini S, Dey MM, Anderson JL (2015) Fish to 2030: The Role and
804 Opportunity for Aquaculture. *Aquaculture Economics and Management*, 19, 282–300.
805 Koblmüller S, Schliewen UK, Duftner N *et al.* (2008) Age and spread of the haplochromine cichlid fishes
806 in Africa. *Molecular Phylogenetics and Evolution*, 49, 153–169.
807 Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., Calcott, B. (2017) PartitionFinder 2: new methods
808 for selecting partitioned models of evolution for molecular and morphological phylogenetic
809 analyses. *Molecular Biology and Evolution*, 34, 772-773.
810 Lowe-McConnell RH (1959) Breeding behaviour patterns and ecological differences between Tilapia
811 species and their significance for evolution within the genus Tilapia (Pisces: Cichlidae). *Proceedings*
812 *of the Zoological Society of London*, 132, 1–30.
813 Matschiner M, Musilová Z, Barth JMI, Starostová Z, Salzburger W Mike Steel M, Bouckaert R (2017)
814 Bayesian phylogenetic estimation of clade ages supports Trans-Atlantic dispersal of cichlid fishes.
815 *Systematic Biology*, 66, 3-22.
816 Mazzuchelli J, Kocher TD, Yang F, Martins C (2012) Integrating cytogenetics and genomics in
817 comparative evolutionary studies of cichlid fish. *BMC Genomics*, 13, 463.

McCann J, Schneeweiss GM, Stuessy TF, Villaseñor JL, Weiss-Schneeweiss H (2016) The Impact of reconstruction methods, phylogenetic uncertainty and branch lengths on inference of chromosome number evolution in American Daisies (*Melampodium*, Asteraceae). *PLoS ONE*, 11, e0162299.

Meade A. (2011) BayesTrees 1.1. Available at: http://www.evolution.rdg.ac.uk/BayesTrees.html

Meier JI, Marques DA, Mwaiko S, Wagner CE, Excoffier L, Seehausen O (2017) Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nature Communications,* 8, 14363.

Meyer BS, Salzburger W (2012) A novel primer set for multilocus phylogenetic inference in East African cichlid fishes. *Molecular Ecology Resources*, 12, 1097–1104.

Meyer BS, Matschiner M, Salzburger W (2017) Disentangling incomplete lineage sorting and introgression to refine species-tree estimates for Lake Tanganyika cichlid fishes. *Systematic Biology*, 66, 531–550.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop, New Orleans, LA, 14 Nov 2010, pp 1–8.

Mmochi AJ (2017) Growth rates of selected *Oreochromis* species cultured at different salinities. World Aquaculture meeting. Abstract.

Moser FN, Rijssel JV, Ngatunga B, Mwaiko S, Seehausen O (2018) The origin and future of an endangered crater lake endemic; phylogeography and ecology of *Oreochromis hunteri* and its invasive relatives. *Hydrobiologica*. doi.org/10.1007/s10750-018-3780-z

Murray AM, Stewart KM (1999) A new species of tilapiine cichlid from the Pliocene, Middle Awash, Ethiopia. *Journal of Vertebrate Paleontology*, 19, 293–301.

Nagl S, Tichy H, Mayer WE *et al.* (2001) Classification and phylogenetic relationships of African tilapiine fishes inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 20, 361–374.

Ndiwa TC, Nyingi DW, Agnese JF (2014) An important natural genetic resource of *Oreochromis niloticus* (Linnaeus, 1758) threatened by aquaculture activities in Loboi Drainage, Kenya. *PLoS ONE* 9(9), e106972.

845 Nevado B, Fazalova V, Backeljau T, Hanssens M, Verheyen E (2011) Repeated unidirectional
846 introgression of nuclear and mitochondrial DNA between four congeneric Tanganyikan cichlids.
847 *Molecular Biology and Evolution*, 28, 2253–2267.
848 Njiru M, Waithaka E, Muchiri M, van Knaap,M, Cowx IG (2005) Exotic introductions to the fishery of Lake
849 Victoria: What are the management options? *Lakes & Reservoirs: Research and Management,* 10,
850 147.
851 Ogilvie HA, Heled J, Xie D, Drummond AJ (2016) Computational performance and statistical accuracy of
852 *BEAST and comparisons with other methods. *Systematic Biology*, 65, 381-396.
853 Ogilvie HA, Bouckaert RR, Drummond A (2017) StarBEAST2 brings faster species tree inference
854 and accuarate estimation of substitution rates. *Molecular Biology and Evolution*, 34, 2101-2114.
855 Pagel M. (2004) Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology*,
856 53, 673–684.
857 Pagel M, Meade A (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-
858 jump Markov chain Monte Carlo. *The American Naturalist,* 167, 808–825.
859 Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language.
860 *Bioinformatics*, 20, 289-290.
861 Penk S, Schliewen U, Altner M, Reichenbacher, B (2018) The earliest record of the Oreochromini
862 (Cichlidae: Pisces) from a Miocene lacustrine deposit in the East African Rift Valley. 5th International
863 Paleontological Congress. Abstract.

864 Philippart J-C, Ruwet J-C (1982) Ecology and distribution of tilapias, p. 15-59. In RSV Pullin and RH
865 Lowe-McConnell (eds.) The biology and culture of tilapias. ICLARM Conference Proceedings 7, 432 p.
866 International Center for Living Aquatic Resources Management, Manila, Philippines.

867 Poletto AB, Ferreira IA, Cabral-de-Mello D *et al.* (2010) Chromosome differentiation patterns during
868 cichlid fish evolution. *BMC Genetics*, 11, 50.
869 Pouyaud L, Agnèse JF (1995) Phylogenetic relationships between 21 species of three tilapiine genera
870 Tilapia, Sarotherodon and Oreochromis using allozyme data. *Journal of Fish Biology*, 47, 26–38.

R Core Team (2018) R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rabosky DL, Santini F, Eastman J, Smith SA, Sidlauskas B, Chang J, Alfaro ME (2013) Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications*, 4: 1958.

Rabosky DL, Chang J, Pascal O, Cowman, PF, Sallan L, Friedman M, Kaschner K, Garilao C, Near TJ, Coll M, Alfaro ME (2018) An inverse latitudinal gradient in speciation rate for marine fishes *Nature*, 559, 392–395.

Rambaut R, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67, 901–904.

Regan C (1920) The classification of the fishes of the family Cichlidae. *Annals and Magazine of Natural History*, 5, 1–71.

Riedel R, Costa-Pierce BA (2005) Feeding ecology of Salton Sea Tilapia (*Oreochromis* spp). *Bulletin of the Southern California Academy of Sciences*, 104, 26–36.

Robinson O, Dylus D, Dessimoz C (2016) Phylo.io: Interactive viewing and comparison of large phylogenetic trees on the web. *Molecular Biology and Evolution*, 33, 2163-2166.

Rognon X, Guyomard R (2003) Large extent of mitochondrial DNA transfer from *Oreochromis aureus* to *O. niloticus* in West Africa. *Molecular Ecology*, 12, 435–445.

Ronquist F, Teslenko M, van der Mark P, *et al.* (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.

Schliewen UR, Klee B (2004) Reticulate sympatric speciation in Cameroonian crater lake cichlids. *Frontiers in Zoology*, 1, 5.

Schluter T, Kohring R, Mehl J (1992) Hyperostotic fish bones ("Tilly bones") from presumably Pliocene phosphorites of the Lake Manyara area, northern Tanzania. *Paläontologische Zeitschrift*, 66, 129–136.

Schluter D (2000) *The ecology of adaptive radiation*. Oxford University Press, Oxford.

Schwarzer J, Misof B, Tautz D, Schliewen U (2009) The root of the East African cichlid radiations. *BMC Evolutionary Biology*, 9, 186.

Seegers L (2008) The fishes collected by G A Fischer in East Africa in 1883 and 1885/86. *Zoosystematics and Evolution*, 84, 149–195.

Seegers L, Tichy H (1999) The *Oreochromis alcalicus* flock (Teleostei: Cichlidae) from Lake Natron and Magadi, Tanzania and Kenya, with description of two new species. *Ichthyological Explorations of Freshwaters*, 10, 97–146.

Seegers L, Sonnenberg R, Yamamoto R (1999) Molecular analysis of the *Alcolapia* flock from lakes Natron and Magadi, Tanzania and Kenya (Teleostei: Cichlidae), and implications for their systematics and evolution. *Ichthyological Explorations of Freshwaters*, 10, 175–199.

Seegers L (2008) The fishes collected by G A Fischer in East Africa in 1883 and 1885/86. *Zoosystematics and Evolution,* 84, 49–95.

Seehausen O, Koetsier E, Schneider MV, Chapman LJ, Chapman CA, Knight ME, Turner GF, van Alphen JM, Bills R (2003) Nuclear markers reveal unexpected genetic variation and a Congolese–Nilotic origin of the Lake Victoria cichlid species flock. *Proc. R. Soc. Lond. B*, 270, 129–137.

Seehausen O (2007) Chance, historical contingency and ecological determinism jointly determine the rate of adaptive radiation. *Heredity*, 99, 361–363.

Seehausen O (2015) Process and pattern in cichlid radiations - inferences for understanding unusually high rates of evolutionary diversification. *New Phytologist*, 207, 304–312.

Shechonge A, Ngatunga BP, Bradbeer SJ, Day JJ, Ford AGP, Kihedu J, Richmond T, Mzighani S, Smith AM, Sweke EA, Tamatamah R, Tyers AR, Turner GF, Genner MJ (2019) Widespread colonisation of Tanzanian catchments by introduced *Oreochromis* tilapia fishes: the legacy from decades of deliberate introduction. *Hydrobiologia,* 832, 235.

Shimodaira, H., Hasegawa, M., 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics 17, 1246– 1247.

Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51, 492–508.

Stöver B C, Müller K F (2010) TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics*, 11, 7.

926 Swofford DL (2000) PAUP*: Phylogenetic analysis using parsimony (* and other methods), version
927 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
928 Tamura K, Peterson D, Peterson N *et al.* (2011) MEGA5: molecular evolutionary genetics analysis using
929 maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology*
930 *and Evolution*, 28, 2731–2739.
931 Thys van den Audenaerde DFE (1968) An annotated bibliography of tilapia (Pisces, Cichlidae).
932 Documentation zoologique No. 14, Musée royal d'Afrique centrale, Tervuren.
933 Trewavas E (1982b) Tilapias: taxonomy and speciation. In: *The Biology and Culture of Tilapias* (eds
934 Pullin R, Lowe-McConnel RH), pp. 3–13. ICLARM Conference Proceedings, Manila, Philippines.
935 Trewavas E (1982) Tilapias: taxonomy and speciation. In: *The Biology and Culture of Tilapias* (eds Pullin
936 R, Lowe-McConnel RH), pp. 3–13. ICLARM Conference Proceedings, Manila, Philippines.
937 Trewavas E (1983) *Tilapiine Fishes of the genera Sarotherodon, Oreochromis and Danakilia*. British
938 Museum (Natural History), London.
939 Turner GF, Robinson RL (1991) Ecology, morphology and taxonomy of the Lake Malawi *Oreochromis*
940 *(Nyasalapia)* species flock. *Ann. Mus. Roy. Afr. Centr. Sc. Zool.*, 262, 23–28.
941 Turner GF (2007) Adaptive radiation of cichlid fish. *Current Biology*, 17, R827–R831.
942 Uyeda JC, Zenil-Ferguson R, Pennell MW (2018) Rethinking phylogenetic comparative methods.
943 *Systematic Biology*, 67, 1091–1109.
944 Van Couvering J (1982) Fossil cichlid fish of Africa. In: *Special Papers in Palaeontology*, pp. 29: 1–103.
945 The Palaeontological Association, London.
946 Vanden Bossche J-P, Bernacsek GM (1990) Source Book for the Inland Fishery Resources of Africa, Vol.
947 1, FAO, Rome.
948 Wagner CE, Harmon LJ, Seehausen O (2014) Cichlid species-area relationships are shaped by adaptive
949 radiations that scale with area. *Ecology Letters*, 17, 583–592.
950 Willis SC, Farias IP, Ortí G. (2014) Testing mitochondrial capture and deep coalescence in amazonian
951 cichlid fishes (Cichlidae: *Cichla*). *Evolution*, 68, 256-268.

952 Yu G, Smith D, Zhu H, Guan Y, Lam TT (2017) ggtree: an R package for visualization and annotation of
953 phylogenetic trees with their covariates and other associated data. *Methods in Ecology and*
954 *Evolution*, 8, 28–36.
955 Yue GH, Lin HR, Li JL (2016) Tilapia is the fish for next-generation aquaculture. *Int J Marine Sci Ocean*
956 *Technol*, 3, 11–13.
957 Zwickl, D. J (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological
958 sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of
959 Texas at Austin.
960
961

Figures

Figure 1. Comparison of the A) nuclear DNA species tree (generated using *BEAST); B) nuclear DNA Bayesian concatenated tree, and C) mitochondrial DNA Bayesian concatenated tree (generated using MrBayes). Figures B + C are compared using Phylo.io software (Robinson et al. 2016). Species that are monophyletic are collapsed. Differences and similarities of both trees are highlighted on the branches, in which the lighter to darker colour indicates the similarity of best matching subtrees between the two trees. Black circles below nodes show >95% BPP support values (see supplementary Figures S1 and S2 for complete trees and support values for BPP and BS). *Coptodon* spp. are removed for clarity.

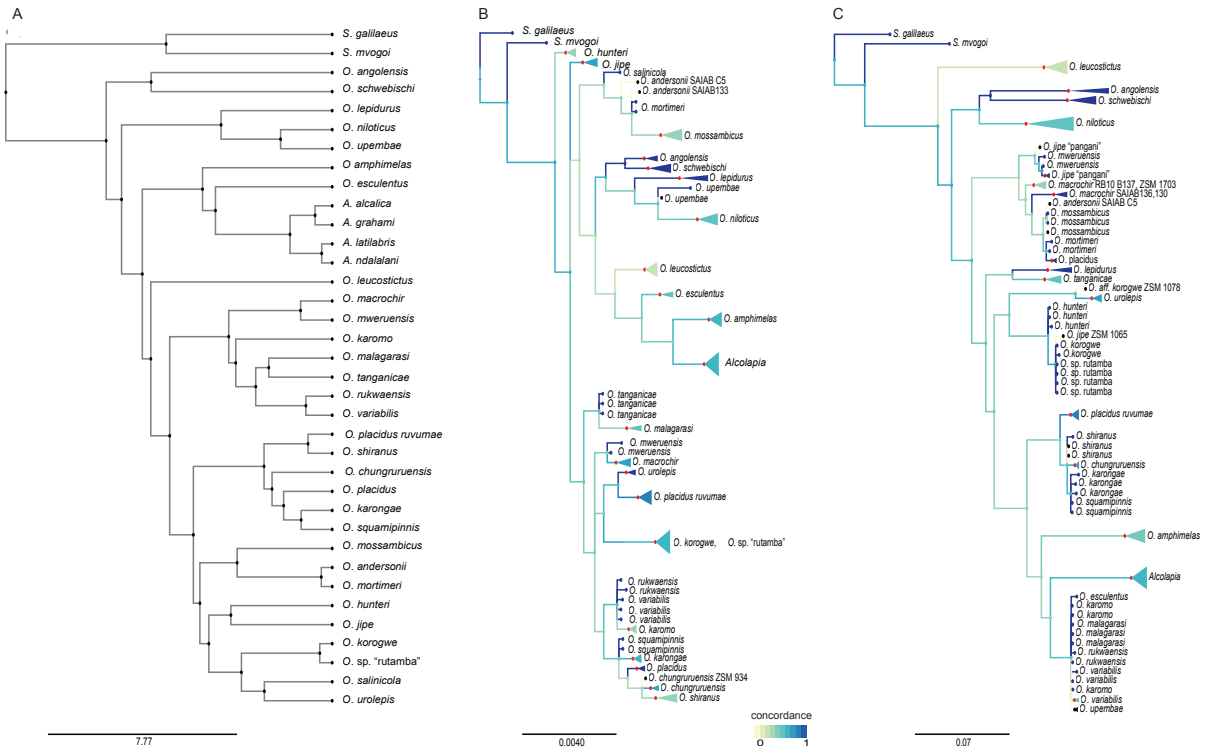
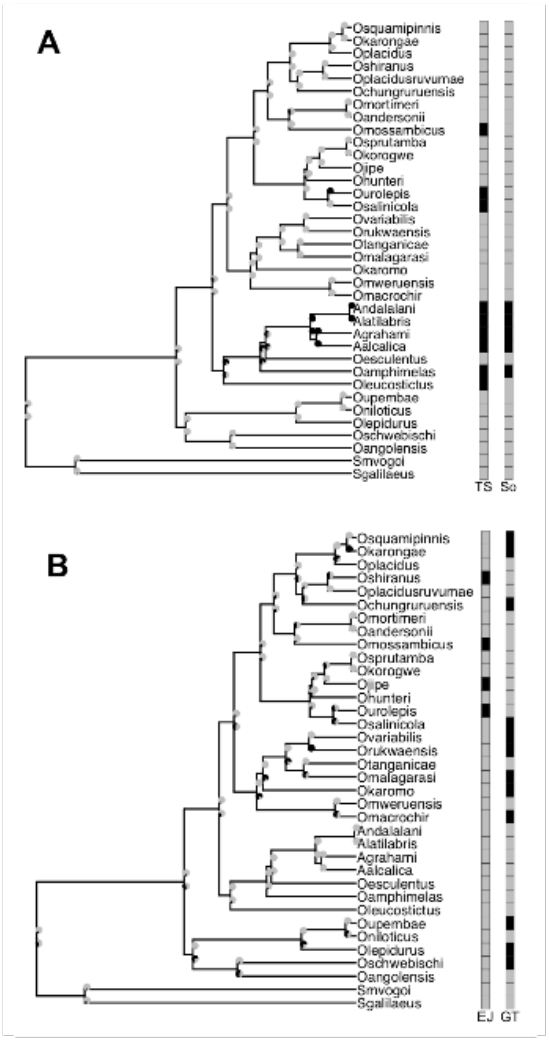


Figure 2. Ancestral state reconstruction from BayesTraits analysis based on the species tree nuclear phylogeny (generated using *BEAST). A) Ancestral state reconstruction of thermal/salinity tolerance (TS) and soda adaptation (So). Colours at tips represent adaptation reported in extant species (listed in Table 1) (black=present; grey=absent). Pie charts at internal nodes indicate probability of presence/absence of ancestor exhibiting adaptation to soda conditions, from BayesTraits analysis. (pie above node: temperature/salinity tolerance ancestral state reconstruction; pie below node: soda adaptation ancestral state reconstruction). B) Ancestral state reconstruction of phenotypic male secondary sexual characteristics: genital tassel (GT) and extended jaw morphology (EJ). Colours at tips represent phenotypic characters in extant species (black=present; grey=absent). Pie charts at internal nodes indicate probability of presence/absence of ancestor exhibiting trait, from BayesTraits analysis (pie above node: extended jaw morphology ancestral state reconstruction; pie below node: genital tassel ancestral state reconstruction).



Species	Natural distribution	Temp°C	Salinity ‰	pH	Ref.
A. alcalica (Hilgendorf 1905)	Natron, TZA	20-42	>40	>10	2,3
A. grahami (Boulenger 1912)	Magadi, Nakuru, KEN	20-42	>40	>10	1,2,3
A. latilabris (Seegers & Tichy 1999)	Natron, TZA	20-42	>40	>10	3
A. ndalalani (Seegers & Tichy 1999)	Natron, TZA	20-42	>40	>10	3
O. amphimelas (Hilgendorf 1905)	Soda lakes, TZA	20-30	58	>9	1,5
O. andersonii (Castelnau 1861)	South-central Africa	18-33	20	nd	1
O. angolensis (Trewavas 1973)	Southern Africa	nd	nd	nd	2
O. aureus (Steindachner 1864)	Eurasia, Africa,	12-32	45	nd	1,2
O. chungruruensis (Ahl 1924)	Chungruru, TZA	nd	freshwater	nd	2
O. esculentus (Graham 1928)	Nile, East African Lakes	23-29	freshwater	nd	1,2
O. hunteri (Günther 1889)	Chala, TZA	nd	nd	nd	2
O. ismailiaensis (Mekkaway 1995)	EGY	nd	nd	nd	
O. jipe (Lowe 1955)	Jipe, Pangani, TZA	nd	nd	nd	2
O. karomo (Poll 1948)	Tanganyika, E. Africa	nd	nd	nd	2
O. karongae (Trewavas 1941)	Malawi, E. Africa	nd	nd	nd	2
O. korogwe (Lowe 1955)	Eastern Africa	nd	freshwater	nd	2
O. lepidurus (Boulenger 1899)	Central Africa	nd	nd	nd	2
O. leucostictus (Trewavas 1933)	Edward, George, UGA	15-38	freshwater	7-9	2
O. lidole (Trewavas 1941)	Malawi, Chungruru, TZA	nd	nd	nd	2
O. macrochir (Boulenger 1912)	S. Africa	18-32	20	nd	1,2
O. malagarasi (Trewavas 1983)	Eastern Africa	nd	nd	nd	2
O. mortimeri (Trewavas 1966)	Southern Africa	19-32	26	nd	1,2
O. mossambicus (Peters 1852)	SE Africa, widely introduced	17-35	>100	nd	1
O. mweruensis (Trewavas 1983)	Congo River system	nd	nd	nd	2
O. niloticus sp 'Bogoria'	Lake Bogoria, KEN	36	nd	7	4
O. niloticus baringoensis (Trewavas 1983)	Baringo, KEN	nd	nd	nd	2
O. niloticus cancellatus (Nichols 1923)	Awash Basin, ETH	17-26	nd	nd	2
O. niloticus eduardianus (Boulenger 1912)	Edward, UGA	nd	nd	nd	2
O. niloticus filoa (Trewavas 1983)	Hot springs, Awash, ETH	32-39	nd	nd	2
O. niloticus niloticus (Linnaeus 1758)	NE Africa	14-32	30	nd	1
O. niloticus sugutae (Daget 1991)	Karpeddo soda springs,	33-38	nd	nd	2
O. niloticus tana (Seyoum 1992)	Lake Tana, ETH	nd	nd	nd	2
O. niloticus vulcani (Trewavas 1933)	Crater lake, Turkana, KEN	nd	nd	nd	2
O. placidus placidus (Trewavas 1941)	Southeastern Africa	nd	freshwater	nd	2
O. placidus ruvumae (Trewavas 1966)	Ruvuma, SE Africa	nd	nd	nd	2
O. rukwaensis (Hilgendorf 1903)	Lake Rukwa, TZA	nd	nd	nd	2
O. saka (Lowe 1953)	Lake Malawi, East Africa	nd	nd	nd	2
O. salinicola (Poll 1948)	Central Africa	nd	25-35	nd	2
O. schwebischi (Sauvage 1884)	West-Central Africa	nd	nd	nd	2
O. shiranus chilwae (Trewavas1966)	Lake Chilwa, MWI	21-37	30	nd	1,2
O. shiranus shiranus (Boulenger 1896)	Lake Malawi and drainage	nd	nd	nd	2
O. spilurus niger (Günther 1894)	Kibwezi River, KEN	19-32	nd	nd	1,2
O. spilurus percivali (Boulenger 1912)	Hot springs, KEN	20-38	'alkaline'	nd	1, 2
O. spilurus spilurus (Günther 1894)	KEN	20-31	nd	nd	2
O. squamipinnis (Günther 1864)	Lake Malawi	nd	nd	nd	2
O. tanganicae (Günther 1893)	Lake Tanganyika	nd	nd	nd	2
O. upembae (Thys van den Audenaerde	Congo river basin	nd	nd	nd	2
O. urolepis urolepis (Norman 1922)	TNZ	25-38	>35	8.4	2
O. variabilis (Boulenger 1906)	Lake Victoria and drainage	23-28	nd	nd	1,2

Tables

Table 1. *Oreochromis* adaptations to soda conditions.

Taxa in bold indicate samples included in the present study (see Table S1). Temperatures and salinity/alkalinity conditions are the maximum at which the species naturally occur. Several studies have shown species are able to tolerate/survive higher levels in laboratory conditions (though fewer have explored successful reproduction at extreme conditions). For the present study, temperature tolerance >35°C, salinity tolerance >30‰, and pH tolerance >pH 9 were considered to represent

elevated environmental tolerance; and tolerance to all three elevated parameters to indicate soda adaptation. Species names in grey were coded as temperature/saline tolerant for ancestral state reconstruction analyses, and those in dark grey were also coded as soda tolerant (see Table S3). References: 1. Philippart & Ruwet 1982; 2. Trewavas 1983; 3. Seegers et al. 1999. 4. Ndiwa et al. 2014. 5. Present study.
nd: minimal data indicating elevated tolerance, presumed not to occur in soda conditions.

Table 2. Substitution models by partition used for the Bayesian and ML analyses.

Partitions	**Best Fitting Model**
nuDNA exon vs. intron	
nuDNA exons	HKY+I+G
nuDNA introns	GTR+I+G
nuDNA loci	
BMP4	HKY+I+G
S7, CCNG1	HKY+G
GAPDHS, TYR, b2m	HKY+I+G
mtDNA	
ND2 pos1, 12S	HKY+I+G
ND2 pos2	TRN+I
ND2 pos3	TRN+G
mtDNA	GTR+I+G